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1 **Spatial and temporal variability of dissolved organic matter molecular composition in**
2 **a stratified eutrophic lake**

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13 **Running Head:**

14 DOM composition in a stratified, eutrophic lake

15 **Key Points:**

16 • O:C increases temporally in a eutrophic lake as a result of photochemical reactions in the
17 epilimnion.

18 • DOM composition varies as a function of depth only when the lake is stratified with
19 higher O:C observed near the bottom of the lake.

20 • DOM composition varies more temporally than it does spatially within the water column.

Abstract

21

22   Dissolved organic matter (DOM) is an intermediate between organic carbon formed by primary

23   producers and CO_2 produced through respiration, making it a key component of the carbon cycle

24   in aquatic ecosystems. Its composition determines the routes of ultimate mineralization. Here we

25   evaluate DOM composition as a function of time and depth in Lake Mendota, a highly

26   productive, eutrophic lake that stratifies in warm months and is located in Madison, Wisconsin,

27   USA. Dissolved organic carbon concentrations and optical properties are presented for 73

28   samples collected at a single location at varying depths within the water column from June to

29   November. A subset of samples are analyzed by Fourier transform-ion cyclotron resonance mass

30   spectrometry (FT-ICR MS) to investigate DOM composition at the molecular level. Temporally,

31   increases in more oxidized formulas are observed in both the epilimnion and hypolimnion. At the

32   surface correlations between DOM formulas and both chlorophyll concentrations and light

33   intensity show that photochemical reactions contribute to DOM oxidation. In the hypolimnion,

34   redox conditions and interactions with sediments likely influence temporal compositional

35   change. Our results show DOM composition varies with depth with more highly oxidized

36   formulas identified deeper in the water column. However, DOM composition varies more

37   temporally than by location within the water column. This work has implications for climate

38   change as DOM photooxidation in lakes represents an understudied flux of CO_2 to the

39   atmosphere. Additionally, lake eutrophication is increasing due to warming temperatures and this

40   data set yields detailed molecular information about DOM composition and processing in such

41   lakes.

Plain Language Summary

43 Dissolved forms of carbon in lakes represent a significant portion of the global carbon pool.

44 Excess phosphorus inputs to lakes cause severe algae blooms which alter other aspects of the

45 lake such as mixing and redox conditions. Lake Mendota, located in Madison, Wisconsin,

46 experiences severe algae blooms in the warm months which cause oxygen depletion deep in the

47 lake. In this study, we considered how changing redox conditions affect the composition of

48 dissolved organic matter (DOM) within the lake. Samples were collected at the surface of the

49 lake and at specific depths between June and November in 2017. Overall, we observed both

50 spatial and temporal variability in DOM composition. Molecular formulas which higher numbers

51 of oxygen atoms were enriched over our sampling period and with depth when the lake was

52 stratified. No changes in DOM composition could be observed when the lake was mixed.

53 Temporal variation was much greater than the spatial variability observed. At the surface, this

54 variability is attributed to reactions occurring due to an input of sunlight.

1. Introduction

55

56   Dissolved organic matter (DOM) is a ubiquitous, naturally occurring substance derived

57   from plant and microbial residues that makes up a significant portion of all organic carbon on the

58   globe [Buffam et al., 2011]. DOM participates in many reactions in aquatic ecosystems. For

59   example, DOM absorbs and blocks ultraviolet light in surface waters [Boyle et al., 2009],

60   forming reactive species that can react with dissolved contaminants [Berg et al., 2019; Boreen et

61   al., 2003; Remucal, 2014], viruses [Silverman et al., 2013], or the DOM pool itself [Gonsior et

62   al., 2009]. The presence of DOM also alters the solubility and availability of potentially harmful

63   species, including toxic metals [Graham et al., 2013; Zhou et al., 2020]. Importantly, the

64   composition of DOM controls its reactivity in all of these processes.

65   DOM composition is driven by differences in source and in extent of environmental

66   processing. However, differentiating between transformation processes is challenging because

67   different processes may yield similar apparent trends and may occur simultaneously. Lakes are

68   an ideal site to differentiate between DOM transformation and source because residence times

69   can be quite long in large lakes, thus allowing ample time for transformation to take place

70   relative to fresh allochthonous inputs. Multiple processes known to alter DOM composition in

71   lakes include microbial transformation [Hertkorn et al., 2002], photochemical transformation

72   [Gonsior et al., 2009] and other physical processes including sorption and dissolution from

73   sediments or particulate phases [Dadi et al., 2017]. Properties specific to particular lakes, such as

74   trophic status, extent of stratification, redox state, and other geochemical parameters, likely also

75   affect composition, although these topics are relatively understudied in DOM composition

76   literature.

77   Here we analyze DOM composition via both ultraviolet-visible (UV-vis) spectroscopy

78 and Fourier transform-ion cyclotron resonance mass spectrometry (FT-ICR MS) in eutrophic

79 Lake Mendota, which is located in Madison, Wisconsin, USA. UV-vis spectroscopy is

80 commonly used to determine bulk properties including the ratio of absorbances at 250 nm to 365

81 nm (E_2:E_3) and the specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) [De Haan and De Boer,

82 1987; Weishaar et al., 2003]. This method is simple and inexpensive, enabling analysis of a large

83 number of samples. FT-ICR MS is a much more time- and resource-intensive approach which

84 allows for molecular-level characterization including weighted averages of H to C (H:C_w), O to

85 C (O:C_w), and double bond equivalents (DBE_w). These results are typically visualized on van

86 Krevelen diagrams which give information about types of compound classes in samples [Kim et

87 al., 2003]. Additionally, the presence of nitrogen and sulfur-containing formulas provide insight

88 into the presence of heteroatoms in DOM [Hertkorn et al., 2008]. Like other methods, FT-ICR

89 MS has some limitations. Specifically, only molecules that are readily ionized can be considered.

90   The thermal stratification Lake Mendota experiences in the summer and fall months

91 allows for the distinct analysis of processes governing DOM transformation near the lake surface

92 and near the bottom. Dissolved oxygen depletion in the bottom layer (i.e., hypolimnion) is driven

93 by eutrophication due to excess nutrients originating mainly from agricultural runoff [Lathrop

94 and Carpenter, 2014]. Dense and frequent cyanobacterial blooms ultimately deliver organic

95 matter to the hypolimnion, creating strong biochemical oxygen demand. When oxygen becomes

96 depleted at depth, often early in the summer, additional terminal electron acceptors become

97 reduced causing build-up of products such as Mn(II), Fe(II), and sulfide. The associated

98 reduction processes are carried out by diverse microbes using DOM as a carbon and energy

99   source [Linz et al., 2018]. Thus, hypolimnetic anoxia is expected to lead to temporal changes in

100   DOM composition.

101   To investigate the transformation of DOM composition in lakes across space and time,

102   we sample a highly eutrophic temperate lake at the surface and as a function of depth from June

103   to November in 2017. Physical and chemical properties of the lake are collected including

104   temperature and concentrations of dissolved oxygen, manganese, iron, and sulfide to thoroughly

105   describe extent of stratification and redox state. DOM is evaluated by its concentration, optical

106   properties, and molecular structure. This unique data set allows for the investigation into in-lake

107   DOM transformation processes throughout the water column during open water conditions.

108   Simultaneous relationships to photo- and biotransformation markers are employed to enable a

109   direct comparison between two key transformation mechanisms at the surface [Herzsprung et al.,

110   2020]. We hypothesized that DOM transformation at the surface of the lake results would result

111   primarily from photochemical reactions that lead to overall oxidation, while variation in DOM

112   composition with depth in the water column would depend on stratification status of the lake.

113

114   **2. Materials and Methods**

115   *2.1 Sample Collection*

116   All samples were collected from Lake Mendota (Madison, Wisconsin, USA), which is a

117   highly eutrophic, temperate lake. This study site was chosen because it is part of the North

118   Temperate Lakes Long-Term Ecological Research (NTL-LTER) program and therefore has a

119   rich set of physical, chemical, and biological data publicly available [Carpenter et al., 2007]. The

120   dominant water source is from the Yahara River and the lake has a mean hydraulic residence

121 time of 4.3 years [Hoffman et al., 2013; Lathrop and Carpenter, 2014]. Surrounding landcover is

122 dominated by cropland and urban land (46.5% and 26.7%, respectively) [Chen et al., 2019].

123   All samples were collected near the NTL-LTER buoy (GPS coordinates: 43.09885,

124 -89.40545), which is near the deepest location in the lake (https://lter.limnology.wisc.edu/data).

125 Surface samples (n = 28) were taken as a composite of the top 12 m of the lake. The top 12 m

126 can generally be considered as the epilimnion, but the location of the thermocline does vary

127 throughout the sampling period (**Figure S1**). Surface samples were collected approximately 1-2

128 times per week from June 2nd through November 3rd in 2017. Depth-discrete samples (n = 45)

129 were collected 1-2 times per month at the exact depth listed as measured by a YSI Exo2

130 multiparameter sonde (YSI Incorporated, Yellow Springs, OH). All water samples were

131 immediately filtered through a 0.22 μm pore-size PES filter and stored in glass bottles in the dark

132 at °4 C. When necessary for analysis, dilutions were made using ultrapure water (18.2 MΩ cm)

133 obtained from a Milli-Q water purification system. A visual representation of a sample inventory

134 for this study is provided in **Figure 1**.

135

136 *2.2 Water Characterization*

137   Geochemical measurements were performed on days when depth-discrete samples were

138 taken and include temperature and concentrations of dissolved oxygen (DO), iron, manganese,

139 and sulfide. Temperature and DO were measured using the YSI sonde. Dissolved concentrations

140 (i.e., able to pass through a 0.45 μm filter) of iron and manganese were quantified by inductively

141 coupled plasma-optical emission spectroscopy (ICP-OES) on a Varian Vista-MPX CCD ICP-

142 OES. Sulfide was quantified spectrophotometrically using the Cline method [Cline, 1969].

143   Concentrations of chlorophyll, temperature, and DO on dates without sample collection were

144   taken from a water quality buoy logging high-frequency measurements that are archived in the

145   NTL-LTER database [Magnuson et al., 2021b]. Chlorophyll concentrations were estimated by

146   relative fluorescent units.

147   Bulk DOM analyses, including measurements of concentration of dissolved organic

148   carbon ([DOC]) and ultraviolet visible (UV-vis) spectra, were recorded for all surface and depth-

149   discrete samples. [DOC] was measured on a Shimadzu total organic carbon analyzer, which was

150   calibrated using known concentrations of potassium hydrogen phthalate as a standard (ACS

151   grade) purchased from Fisher Scientific. Historical [DOC] measurements were obtained from the

152   NTL-LTER database [Magnuson et al., 2021a]. For a more accurate comparison to our surface

153   samples, any historical measurements that are made above 12 m on a single day were averaged

154   together. The average [DOC] for each day occurring within the window of time included in our

155   2017 samples (87 - 241 days after ice-off) were plotted versus days since ice off of the given

156   year. Slope directions were evaluated using Kendall rank correlations with a 95% confidence

157   interval. Historical sampling frequency depended on the year but was approximately monthly.

158   UV-vis spectra were collected via a Shimadzu 2401PC Recording Spectrophotometer

159   with 1 nm intervals between 200 – 800 nm. All spectra were collected in 1 cm cuvettes, blank

160   subtracted to Milli-Q water, and the average absorbance from 700 - 800 nm was also subtracted

161   to correct for any light scattering. The optical property E_2:E_3 was calculated as the ratio of the

162   absorbance at 250 nm to 365 nm and specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) as the

163   absorbance at 254 nm divided by [DOC] [De Haan and De Boer, 1987; Weishaar et al., 2003].

164   *2.3 Mass Spectrometry Analysis*

165   A subset of the depth-discrete samples was analyzed via Fourier transform-ion cyclotron

166   resonance mass spectrometry. Sixteen samples were selected to span a variety of dates, depths,

167   and observations of optical properties since previous work has demonstrated that changes in FT-

168   ICR MS spectra correlate with optical parameters [Berg et al., 2019; Remucal et al., 2020;

169   Maizel et al., 2017c]. DOM was extracted using solid phase extraction and diluted in 50:50

170   acetonitrile:water using methods described elsewhere [Dittmar et al., 2008]. Mass spectra were

171   collected using a SolariX XR 12T FT-ICR mass spectrometer (Bruker) with a Triversa

172   NanoMate sample delivery system (Advion) operating in negative mode with electrospray

173   ionization. m/z values ranging form 200 – 600 were considered. All *m/z* peaks with signal to

174   noise > 3 were exported and considered for matching. Some results of this type of analysis are

175   particularly sensitive to the instrument and instrumental parameters [Hawkes et. al, 2020]. We

176   combat this issue by analyzing all samples back to back on the same day using the same

177   instrumental parameters.

178   Raw data were linearly calibrated using 10 known formulas ranging from 224. 03209 –

179   600.205424 and commonly found in DOM as described previously [Maizel et al., 2017b].

180   Potential formula masses considered included $^{12}C_{1\text{-}100}{}^{13}C_{0\text{-}1}H_{1\text{-}100}O_{0\text{-}80}N_{0\text{-}2}S_{0\text{-}1}P_{0\text{-}1}$. Combinations of

181   heteroatoms allowed included $N_1S_0P_0$, $N_2S_0P_0$, $N_1S_1P_0$, $N_2S_1P_0$, $N_0S_1P_0$, and $N_0S_0P_1$ to limit the

182   potential mass list while maximizing the number of formulas reliably matched. Identified

183   formulas were required to have < 0.2 ppm error between the detected mass and the actual mass

184   of the formulas. Additionally, identified formulas were required to be part of a homologous

185   series (+ CH_2 or CH_4 vs. O) with at least three members [Koch et al., 2007]. Overall in the data

186   set, 6358 unique formulas were identified. Weighted averages for bulk properties (i.e., $H:C_w$ and

187    $O:C_w$) were calculated by summing values for individual formulas multiplied by their weighted

188 intensities. Spearman rank analyses were used to compare relative formula intensities to location

189 within the water column and other parameters. For correlations to depth, the formulas were

190 required to be identified in at least 15 of the samples. Prior to calculation of the correlations for

191 chlorophyll and light intensity, the data set was subset to only include formulas that were

192 commonly identified across the group. Furthermore, the preparatory step of intersample ranking

193 was performed where each formula within a sample is given a rank based on its relative intensity

194 compared to the relative intensities of the other formulas identified in the sample.

195 *2.4 Solar Radiation Modelling*

196 The "Simple Model of the Atmospheric Radiative Transfer of Sunshine" (SMARTS) was

197 used to estimate irradiation at in Madison, WI (43.1097º N, 89.4206º W) at noon on August 11th,

198 September 8th, September 21st, October 4th, and November 3rd of 2017. Intensities were summed

199 from 280 – 500 nm [McConville et al., 2017; Remucal and McNeill, 2011].

200 **3. Results and Discussion**

201 *3.1 Lake Overview*

202 In 2017, ice-off (i.e., the first date open water is observed on the lake) was declared for

203 Lake Mendota on March 8th. Our first surface sample was collected on June 2nd, 87 days after ice-

204 off. The lake is already thermally stratified by the date of our first depth-discrete sample (June

205 29th) and mixes before the last depth-discrete sampling date (November 3rd; **Figures 1** and **S1**).

206 [DOC] ranges from 4.12 – 7.07 mg-C L^{-1} in all samples collected during this study (**Table S7**),

207 which is within the range observed in the historical NTL-LTER database [Magnuson et al.,

208 2021a]. The optical properties SUVA$_{254}$ and E$_2$:E$_3$ range from 1.33 – 2.14 L mg-C^{-1} m^{-1} and 7.86

209   $-$ 10.18 L mg-C^{-1} m^{-1}, respectively (**Table S7**). The relatively low SUVA$_{254}$ and high E$_2$:E$_3$ values

210   are indicative of DOM from autochthonous sources or allochthonous DOM that has undergone

211   extensive environmental processing [Bai et al., 2017a; Berg et al., 2019; Brown et al., 2004;

212   Gonsior et al., 2009; Maizel et al., 2017a].

213

214   *3.2 Temporal Variation at the Surface*

215   The variation in DOM concentration and composition in the upper mixed layer of Lake

216   Mendota provide insight into the dominant mechanisms responsible for shaping the DOM pool

217   across an ice-free season. Since both microbial processing and photochemical reactions occur

218   simultaneously, considering each process individually is an oversimplification. However, we can

219   make inferences about which mechanism is most likely responsible for the observed changes

220   based on correlations to other variables that change on a seasonal scale.

221   Integrated surface samples show DOM concentration and especially DOM composition

222   vary over the sampling period. Although the temporal variation in [DOC] is not significant (p =

223   0.30; **Figure 2a**; **Table S7**), a negative slope with time is observed. Decreasing [DOC] has also

224   been observed historically in Lake Mendota over the same dates for 14 of the past 22 years

225   between 1996 and 2017 but similarly without significant correlation (**Table S8**). We observe a

226   significant decrease in A$_{254}$ over the sampling period (p = 7.11 x 10^{-3}; **Figure 2b**; **Table S7**). This

227   trend means that the relative amount of light absorbing moieties in DOM decrease over time, but

228   that this is not solely attributable to overall decreases in [DOC]. This inferred shift in DOM

229   composition is supported by the significant increase in E$_2$:E$_3$ (p = 2.13 x 10^{-8}; **Figure 2d**; **Table**

230   **S7**), corresponding to a decrease in DOM apparent molecular weight. However, no temporal

231   trend is detected for SUVA$_{254}$ likely because decreases in absorbance are cancelled out by [DOC]

232 variability (**Figure 2c**; **Table S7**). Other studies show similar seasonal increases in $E_2:E_3$ as well

233 as decreases in $SUVA_{254}$ in a eutrophic lake in Sweden [Müller et al., 2014] and in freshwaters in

234 China [Song et al., 2013].

235   The increase in $E_2:E_3$ with time is consistent with both photooxidation and microbial

236 alteration of DOM [Helms et al., 2008; Larson et al., 2007; Sharpless et al., 2014]. Autotrophic

237 organisms living near the surface of the lake produce DOM molecules that are relatively simple

238 and do not absorb light at long wavelengths (e.g., carboxylic acids, amino acids, and peptides)

239 [Hertkorn et al., 2002; Kamjunke et al., 2017; Valle et al., 2018]. For example, incubations

240 isolating DOM produced by phytoplankton show that the DOM is especially small in size [Zhang

241 et al., 2013] and readily available to heterotrophic microbes [Bertilsson and Jones, 2003]. In

242 Lake Mendota, this DOM is unlikely to contribute to measured [DOC] because it is so rapidly

243 consumed. Alternatively, photochemical reactions can break down chromophoric DOM,

244 particularly of allochthonous origin, directly or form photochemically produced reactive

245 intermediates (PPRI) that degrade DOM into smaller DOM molecules that absorb less light

246 [Bade et al., 2007; Bittar et al., 2015; Brinkmann et al., 2003; Helms et al., 2008, 2013, 2014;

247 Kujawinski et al., 2004; Lønborg et al., 2016; Spencer et al., 2009]. Photooxidation can occur

248 both completely to form carbonate species or incompletely to form more oxidized DOM; a

249 combination of both is consistent with our observations. Both photochemical processes could

250 result in increased $E_2:E_3$ and decreased A_{254} [Bittar et al., 2015; Brinkmann et al., 2003; Cory and

251 Kling, 2018; Helms et al., 2008, 2013, 2014; Hur et al., 2011; Kujawinski et al., 2004; Lønborg

252 et al., 2016; Sharpless et al., 2014; Spencer et al., 2009], however only complete oxidation

253 results in [DOC] decreases. Since significant changes in [DOC] are not observed, neither

254   autotrophic inputs nor complete oxidation to CO_2 are likely driving the observed differences in

255   DOM composition over the summer. Instead, incomplete oxidation is likely the dominant

256   mechanism if photochemical reactions are responsible for the change in DOM composition

257   observed in Lake Mendota. It is possible that DOM sinks are equal to DOM sources, but is not

258   possible to test this hypothesis with bulk analyses alone.

259   A subset of the depth-discrete samples collected from the epilimnion (n = 5) are analyzed

260   by FT-ICR MS and can be used to evaluate changes in the molecular composition of DOM at the

261   lake surface, providing more detailed information than bulk UV-vis spectroscopy measurements

262   (**Figures S5**, **S8**, **S10**, **S12**, and **S16**). $H:C_w$ values of identified formulas range from 1.17 – 1.24

263   and decrease significantly over the course of the summer (p = 1.56 x 10^{-2}; **Figure 2e**; **Table S4**).

264   This increase in aromaticity is accompanied by an increase in $O:C_w$ (range = 0.49 – 0.55; p =

265   3.06 x 10^{-3}; **Figure 2f**). These ranges in weighted averages derived from FT-ICR MS data are

266   consistent with DOM that is largely microbially-derived and/or has undergone extensive

267   environmental processing [Brown et al., 2004; Hawkes et al., 2020]. The large variation in these

268   parameters, and particularly in $O:C_w$, is especially noteworthy given that the samples were taken

269   from the same location in a single lake. In fact, the range in $O:C_w$ in this study is approximately

270   the same range observed across the entire St. Louis River and Estuary which includes upstream

271   samples dominated by terrestrial inputs and water from oligotrophic Lake Superior [Berg et al.,

272   2019]. DBE_w and % CHO appear to increase while % N-containing and % S-containing formulas

273   appear to decrease over the sampling period, but no significant trends are observed for the five

274   samples (p = 0.161, 0.168, 0.111, and 0.157, respectively; **Figure S18**).

275   Molecular data make it possible to track temporal changes of individual identified

276   formulas. Lipid- and protein-like compounds show a relative decrease over the course of the

277   summer indicating their consumption rates are greater than production rates (**Figure 3a**). This

278   results in the overall decrease in H:C$_w$ observed at the surface (**Figure 2e**). Formulas with high

279   O:C and a range of H:C become more intense relative to other formulas over our sampling period

280   (**Figure 3b**); these aromatic, tannin-like formulas cause an increase in O:C$_w$ (**Figure 2f**).

281   The changes in DOM composition in the surface of Lake Mendota could be attributable

282   to microbial processing. We use the term "microbial" in this context to refer to both autotrophs

283   and heterotrophs, meaning that DOM can be created through primary production but can also be

284   processed through respiratory mineralization and release of dead cell components. Phytoplankton

285   are known to produce relatively simple molecules such as peptides and small carboxylic acids

286   [Meon and Kirchman, 2001]. Although these small molecules are outside the 200 – 800 *m/z*

287   range of our FT-ICR MS instrument, high-resolution mass spectrometry has been used to study

288   other larger phytoplankton-derived DOM and shows that products that are detected are most

289   similar to protein- and lipid-like compounds [Mangal et al., 2016]. Many of these formulas are

290   especially bioavailable for consumption by heterotrophic microbes [D'Andrilli et al., 2015] and

291   thought to undergo phototransformation into refractory proteinaceous compounds [Goldberg et

292   al., 2015]. While the formulas that are degraded most quickly might never be detected because of

293   rapid turnover and/or analytical limitations, the net decrease in highly saturated formulas is

294   consistent with net consumption of these types of formulas (**Figure 3a**) and overall enrichment

295   in more biologically recalcitrant, low molecular weight compounds. This argument is in line with

296 the microbial carbon pump framework used to explain recalcitrant DOM accumulation in the

297 ocean [Jiao et al., 2010; Zhang et al., 2018].

298   Our observations may also be explained by photochemical oxidation. DOM photolysis

299 produces PPRI including singlet oxygen ($^1O_2$) and hydroxyl radical ($^{\bullet}OH$) that may react with

300 phytoplankton-derived DOM via incomplete pathways that generate oxidized DOM [Ward and

301 Cory, 2016]. $^1O_2$ is a selective oxidant that preferentially oxidizes nucleophilic compounds and

302 can be quenched by DOM [Hessler et al., 1996; Yang et al., 2013]. $^{\bullet}OH$ also reacts quickly with

303 aromatic rings via oxygen addition to generate more oxidized products, which is consistent with

304 the observed increases in O:C_w (**Figure 2f**) and the shift toward more oxidized lignin- and

305 tannin-like formulas (**Figure 3b**) [Remucal et al., 2020]. Thus, the observed changes in weighted

306 averages derived from FT-ICR MS data could be attributed either microbial or photochemical

307 processing.

308   It is important to recognize that microbial and photochemical processing occur

309 simultaneously in the surface of the lake and that changes in DOM composition due to one

310 process could impact the other process. For example, both increases and decreases in bacterial

311 growth have been observed as a result of photochemical alteration of DOM [Cory and Kling,

312 2018; Tranvik and Bertilsson, 2001]. While these divergent trends may be attributable to the

313 original source of the DOM [Tranvik and Bertilsson, 2001], they also may be an artifact of the

314 length of the experiment and whether or not microbial communities have been allowed time to

315 adapt to the photoproduced DOM [Cory and Kling, 2018]. In Lake Mendota, the microbial

316 community composition has been shown to change on daily to weekly time scales during the

317 summer [Kara et al., 2013; Shade et al., 2007], but the effects of this change on DOM cycling

318 have not been studied. In less productive arctic systems, shifts in heterotrophic community

319 composition precede increases in DOM degradation rates [Cory and Kling 2018], underscoring

320 the tight integration of DOM production, photodegradation, and biological transformations.

321   To differentiate between these two transformation mechanisms (i.e., microbial processing

322 versus photo-altered DOM), we use an approach originally described in Herzprung *et al*. [2020]

323 to determine formulas produced and consumed by microbial and photochemical processes based

324 on correlations with chlorophyll and solar radiation, respectively (**Figure 4**; **Table S3**). Only

325 formulas identified in all surface samples are considered for this analysis and rho correlations are

326 only included for those with $p < 0.05$ for correlations with either chlorophyll or light intensity.

327 Chlorophyll and light intensity values on our sampling dates are listed in **Table S3**. Formulas are

328 classified into five reaction types including photodegraded compounds (negative correlation with

329 radiation, n = 75), photoproducts (positive correlation with radiation, n = 494), microbially

330 degraded compounds (negative correlation with chlorophyll, n = 88), microbially derived

331 products (positive correlation with chlorophyll, n = 14), and nonreactive (no significant

332 correlation with either parameter, n = 1060; **Figures 4** and **S20**). No formulas have significant

333 correlations with both parameters. Thus, formulas that decrease in their relative abundance are

334 classified as "degraded", while the formulas that increase in their relative abundance are

335 classified as "products".

336   Classified formulas fall within distinct regions of the van Krevelen diagram.

337 Photodegraded compounds (**Figure 4a**) are depleted in O and are highly saturated. The large

338 number of formulas classified as photoproducts have relatively high O:C ratios and fall within

339 the tannin- and lignin-like regions of the diagram (**Figure 4b**), which supports the hypothesis

340 that partial photochemical reactions drive DOM composition changes at the surface. The

341 accumulation of these formulas is noteworthy because they are also efficient at photochemically

342 producing $^{\bullet}OH$, but not other PPRI like ^{3}DOM and $^{1}O_2$ [Berg, et al., 2019], suggesting that the

343 photochemical reactivity of DOM likely changes over the summer.

344   In contrast to photochemical reactivity, a relatively small number of formulas detected by

345 FT-ICR MS are susceptible to biotransformation based on correlations with chlorophyll (**Figure**

346 **4c**; **Figure S20**). However, we recognize that our sampling approach likely misses the most

347 labile formulas which are consumed as quickly as they are produced and not part of the more

348 recalcitrant DOM pool measured here. The formulas that are classified as microbially degraded

349 (**Figure 4c**) are generally the same types that are classified as photodegraded (**Figure 4a**).

350 Therefore, in Lake Mendota, photochemical reactions would likely make DOM less bioavailable,

351 consistent with incubation studies [Cory and Kling, 2018; D'Andrilli et al., 2015]. Collectively,

352 these data suggest that the DOM in Lake Mendota is mostly phytoplankton-derived and/or highly

353 processed (i.e., refractory) based on low $SUVA_{254}$ and high $E_2{:}E_3$ and $H{:}C_w$ values, but that the

354 DOM detected by FT-ICR MS is produced or altered mainly through photochemical reactions

355 over the course of our sampling period.

356 *3.3 Variation with Depth*

357   Geochemical and physiochemical parameters measured in the depth-discrete samples

358 show that redox conditions are altered as Lake Mendota undergoes stratification. Temperature

359 and DO data indicate that Lake Mendota is stratified on every depth-discrete date except

360 November 3rd (**Figures 5** and **Figures S21-S26**). Once stabilized, the thermocline is observed

361 around 10 – 15 m into the water column (**Figure S1**). Anoxia, as determined by DO

362   measurements, is observed in the hypolimnion by the beginning of June and remains until the

363   lake mixed in late October (**Figure 1**). The presence of reduced forms of iron and manganese, as

364   well as sulfide, indicate that alternative electron acceptors are used when DO is depleted (**Figure**

365   **S21-S26**; **Table S2**). Dissolved iron (11.4 – 114 µg L^{-1}) is detected in all samples in the

366   hypolimnion except samples collected on November 3rd after the lake mixes. Dissolved

367   manganese concentrations range from 0 – 263 µg L^{-1}. Later in the season, Mn(II) is observed at

368   more shallow depths likely due to anoxic conditions existing near the thermocline. Sulfide is

369   only detected when DO is depleted and concentrations increase later in the summer due to the

370   consumption of other more thermodynamically favorable electron acceptors. Up to 5.4 mg L^{-1} of

371   sulfide is measured (**Table S2**). Once the lake is mixed in late October, Fe(II), Mn(II), and

372   sulfide are not present above detection limits likely due to oxidation by O_2 and resorption to

373   sediments [Krueger et al., 2020].

374   [DOC] varies within the water column when the lake is stratified but does not change

375   consistently as a function of depth (**Figures 5c** and **Figures S21-S26**). Sharp changes in [DOC],

376   including decreases of around 2 mg-C L^{-1} over a meter, are observed around the thermocline

377   when the lake is stratified. This is noteworthy because the lowest [DOC] is observed right around

378   the thermocline, which is where redox conditions change most rapidly with depth and microbial

379   activity is usually high [Peterson et al., 2020]. DOM may also co-precipitate with redox active

380   metals, likely manganese, to form particulates and thus carbon in the dissolved phase decreases.

381   In fact, spikes in particulate Mn are observed around the thermocline in this lake in the same year

382   [Peterson et al., 2020]. Changes in concentrations of DOM and metals due to complexation and

383   precipitation reactions have been observed in other systems such as a creek bank[65] and an

384   experimental drinking water treatment system.[66] In contrast, in November when the lake is

385   mixed, [DOC] is constant with depth, as expected (**Figure 5c**).

386   Bulk DOM composition, as determined using optical measurements, also varies with

387   depth during stratification. $SUVA_{254}$ is consistently lower and E_2:E_3 is consistently higher in the

388   epilimnion compared to the hypolimnion when the lake is stratified (**Figures 5** and **S21-S26**).

389   Similar evidence of DOM that is relatively more aromatic and higher in molecular weight being

390   enriched in the hypolimnion has been observed and attributed to decreasing aromaticity due to

391   photobleaching at the surface of the artificial Lake Salto [Bracchini et al., 2006]. No variability

392   in optical properties are observed after the lake mixes (**Figures 5d-5f**). Interestingly, once the

393   lake mixes, values for $SUVA_{254}$ are in the middle of the range observed over the summer (**Figure**

394   **5d**). In contrast, values for E_2:E_3 are the highest of the season after the lake mixes (**Figure 5e**).

395   This agrees with the observations made at the surface of the lake that E_2:E_3 increases over the

396   sampling period but $SUVA_{254}$ is constant (**Figure 2**).

397   Relative amounts of heteroatoms also varies with depth both when the lake is stratified

398   and when it is mixed (**Figures 6a-6c**; **Tables S5-S6**). Ranges of % CHO, % N-containing, and %

399   S-containing formulas vary from 38.7 – 49.9%. The percentage of CHO-only containing

400   formulas decreases in the hypolimnion on each of the five days considered (**Figure 6a**), while the

401   percentages of N- and S-containing formulas increase (**Figures 6b-6c**). The difference between

402   heteroatom-containing formulas and CHO-only formulas is largest in the samples taken later in

403   the season (i.e., October 4th and November 3rd), which suggests that the distribution of

404   heteroatom-containing DOM does not depend on stratification since the lake mixed before

405   November 3rd.

19

406   The decrease in percentage of CHO-only formulas at the bottom of the lake is due to

407   increases in heteroatom-containing formulas since the total number of formulas identified does

408   not account for these differences (**Figure 6a; Table S6**). Increases in S-containing formulas may

409   be due to nucleophilic attack by sulfide on DOM [Sleighter et al., 2014; Poulin et al., 2017;

410   Schmidt et al., 2017; Vairavamurthy and Mopper, 1987]. The buildup of sulfide under anoxic

411   conditions (**Table S2**) creates greater opportunity for this type of reaction. Linear correlations

412   between concentrations of sulfide and % S-containing formulas have been reported previously

413   [Poulin et al., 2017]. Here, the highest % S-containing formulas are observed when higher sulfide

414   concentrations are detected, but the trend is not linear (**Figure S27**). However, nucleophilic

415   attack cannot be the only formation mechanism since a range of % S-containing formulas is

416   observed even when sulfide is not detected, particularly at the lake surface. Additionally, the S-

417   containing formulas observed in this data set preferentially fall within relatively saturated regions

418   of the van Krevelen diagram which could be evidence of a biological pathway for their formation

419   (**Figures S3**-**S17**) [Sleighter et al., 2014]. In contrast, N-containing formulas make up

420   approximately the same space on the van Krevelen diagrams as the DOM pool as a whole at all

421   depths and therefore may be formed via abiotic addition of N-containing nucleophiles (**Figures**

422   **S3**-**S17**) [Sleighter et al., 2014].

423   Changes in DOM molecular composition on individual days can be used to investigate

424   how composition varies temporally and by depth when the lake is stratified and mixed. $H:C_w$ and

425    $O:C_w$ vary by date much more than they vary with depth, with $H:C_w$ values decreasing and $O:C_w$

426   values increasing over the sampling period (**Figures 6d-6e**; **Table S4**). On each individual day

427   when the lake is stratified, $H:C_w$ is higher in the epilimnion ($p = 0.049$) and $O:C_w$ appears higher

428   in the hypolimnion although the relationship is not statistically significant (p = 0.096). The

429   opposite trend is observed on November 3rd when the lake is mixed. This observation was also

430   made after a mixing event in a boreal lake in Sweden [Gonsior et al., 2013]. DBE_w also appears

431   to vary more temporally than spatially although less consistently than $H:C_w$ or $O:C_w$ (**Figure 6f**).

432   Like the optical properties, changes in molecular composition are observed at the

433   thermocline. Four samples collected on August 11th are analyzed by FT-ICR MS, including one

434   sample above the thermocline (i.e., 11.5 m) and the sample immediately below the thermocline

435   (i.e., 13.2 m). On this day, DOM sampled just below the thermocline has lower $H:C_w$ and higher

436    $O:C_w$ and DBE_w than at any other location in the water column. In terms of heteroatoms, the

437   percentages of % CHO-only and % N-containing formulas increase while the percent of % S-

438   containing formulas decreases (**Figure 6**) near the thermocline. If co-precipitation of DOM with

439   metals in the thermocline is responsible for loss of [DOC] as discussed above, these results

440   suggest preferential removal of aromatic and S-containing DOM. Subsequent dissolution below

441   the thermocline would result in the increase of $O:C_w$ and decrease in $H:C_w$ at 12 m where [DO] is

442   depleted, which matches our observations. While this observation is only based on one sampling

443   date, preferential removal of highly oxidized DOM has been reported with other metal oxides in

444   a lake and in a water treatment system [Barazesh et al., 2018; Riedel et al., 2013].

445   Specific formulas identified within DOM by FT-ICR MS can be correlated to location

446   within the water column and with bulk optical properties. These analyses are especially

447   informative because they allow for probing which individual molecular formulas are responsible

448   for the trends in weighted averages described above. Correlating relative intensities of formulas

449   commonly identified in the depth-discrete samples shows that, in general, formulas with higher

450   O:C ratios are enriched in the hypolimnion (**Figure 7**). There is also a slight dependence on the

451   H:C ratio, with more aliphatic formulas enriched near the surface of the lake. The same general

452   pattern of correlations is observed for relative intensities correlated to DO (**Figure S19**).

453   However, it is important to note that there is only one sampling day where DO is not dependent

454   on depth (i.e., in November when the lake is mixed), so it is unclear whether or not this single

455   day is enough to disrupt the overall trend.

456   O:C_w ratios increase with depth while the lake is stratified, as well as temporally over our

457   sampling period at all depths (**Figures 6e** and **7b**). While increases in oxygenated formulas at the

458   surface are likely attributable to photochemical reactions (**Figure 4**), other processes must be at

459   play in the hypolimnion since light is fully attenuated higher in the water column. Highly

460   oxygenated formulas that are likely polyphenolic- and tannin-like are enriched in the

461   hypolimnion of other lakes including a mesotrophic pre-dam reservoir [Dadi et al., 2017] and a

462   stratified, humic lake in Sweden [Gonsior et al., 2013]. These types of formulas have been found

463   in sediments and could be enriched by desorption at the bottom of lakes [Dadi et al., 2017;

464   Gonsior et al., 2013; Herzsprung et al., 2017; Sleighter et al., 2014; Riedel et al., 2013; Schmidt

465   et al., 2011, 2017; Valle et al., 2020; Yang et al., 2014]. In fact, highly oxygenated formulas are

466   preferentially incorporated into redox active metal oxides, which then dissolve more readily

467   under anoxic conditions [Barazesh et al., 2018; Linkhorst et al., 2017; Lv et al., 2016; Riedel et

468   al., 2013; L. Yang et al., 2014]. This phenomenon may explain why O:C ratios increase at the

469   bottom of Lake Mendota temporally as more minerals undergo reductive dissolution in the

470   anoxic conditions. Alternatively, heterotrophic metabolism may consume the smaller, simpler

471 DOM leaving the larger, more oxidized material left in solution. More work is needed to

472 distinguish between these two processes.

473 **4. Conclusions and Implications**

474 By focusing on a single location in a single lake, we are able to concurrently investigate

475 how a combination of photochemical, biological, geochemical, and physical processing impacts

476 DOM composition. At the surface of the lake, A_{254} decreases and $E_2:E_3$ increases, indicating a

477 shift to DOM that is smaller in molecular weight and a decrease in light absorbance by DOM

478 which is not attributable to decreases in [DOC] alone. While these observations could be

479 consistent with either microbial or photochemical processing, our molecular level analysis allows

480 for the conclusion that DOM transformation at the surface of Lake Mendota is primarily driven

481 by photochemical processes.

482 DOM composition also varies by depth within the water column with larger, more

483 aromatic DOM enriched at the anoxic bottom of the lake during stratification. After lake mixing,

484 [DOC] and optical properties can no longer be used to differentiate between DOM collected at

485 different depths. However, differences in heteroatom content of the DOM are still observed via

486 molecular level analysis. Combined, these results show that single grab samples taken from a

487 dynamic lake such as Lake Mendota should not be considered representative of all seasons nor

488 all locations within the water column.

489 While variability both temporally and spatially within the water column was observed,

490 the temporal variation was much greater. This has implications for researchers designing

491 sampling campaigns in this lake and likely other stratified lakes. Given these results, it would be

492 a much better use of time and resources to sample with more temporal resolution than it would

493 be to sample at multiple depths within the lake if researchers are interested in the DOM.

494   The effect of eutrophication on DOM composition is vital to understanding DOM in our

495 changing climate. Eutrophication will affect an increasing number of water bodies as

496 temperatures increase and more runoff makes its way into the surface water [Sinha et al., 2017].

497 Understanding overall water quality including DOM composition is important to mitigate

498 adverse effects of eutrophication including the production of cyanotoxins and hypoxia in water

499 bodies [Hao et al., 2020; Song et al., 2012]. Increases in [DOC], also referred to as browning

500 waters, is commonly reported, particularly in European freshwaters, and while the exact

501 mechanisms are still being debated, most link to a changing climate [Evans et al., 2006; Freeman

502 et al., 2004; Worrall et al., 2004]. Interestingly, this phenomenon is not observed in all lakes

503 including other NTL-LTER lakes in Wisconsin [Jane et al., 2017]. Both browning and

504 eutrophication are expected to alter photochemistry in natural systems and may cause shifts in

505 DOM composition that are difficult to predict [Vione and Scozzaro, 2019].

506   The distribution of DOM with varying composition throughout the water column is

507 significant for many reasons. DOM composition is highly linked to bioactivity, and therefore any

508 chemical or physical processes that affect DOM are also likely to affect microbial populations

509 and vice versa [Bowen et al., 2020; Guerrero-Feijóo et al., 2017; Lønborg et al., 2016; Ward et

510 al., 2017]. The relationship between photochemistry and microbial metabolism is complicated,

511 however, and both increases and decreases in bioavailability have been reported after DOM

512 irradiation [Ward et al., 2017]. While not explicitly tested here, the fact that the same types of

513 formulas are classified as photodegraded and microbially degraded suggests competition between

514 photo- and biotransformation likely exist. The effect of stratification on DOM composition could

24

515 also have important implications for greenhouse gas fluxes, particularly after the lake mixes

516 when DOM not yet exposed to sunlight reaches the surface. For example, fresher, less processed

517 DOM from thawing arctic permafrost layers has been shown to be particularly photo- and bio-

518 labile [Ward et al., 2017]. Thus, the effects of lake stratification on DOM composition may have

519 important implications for climate change.

520

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Data Availability Statement

534 Supporting Information S1 including additional information about methods, tabulated

535 data, and figures is provided. Raw data are made publicly available as .csv files through the

536 NTL-LTER repository (FT-ICR MS:

537 https://doi.org/10.6073/pasta/c0bd1ca5de343dc93c74395d21bf3fc6; UV-vis spectra:

538 https://doi.org/10.6073/pasta/d668dcf76d98cf7f94dbb0ec2fd41a51; no registration required;

539 licensing: CC-BY-NC).

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542

Figure Captions

Figure 1. Dissolved oxygen (DO) profile heatmap for Lake Mendota for June – November 2017 based on DO measurements taken by the NTL-LTER research buoy. Points indicate sampling dates and depths of samples collected for DOM analysis.

Figure 2. a) [DOC], b) $SUVA_{254}$, c) $E_2:E_3$, and d) A_{254} for integrated epilimnion samples and e) $H:C_w$ and f) $O:C_w$ of identified formulas detected by FT-ICR MS in the surface depth-discrete samples.

Figure 3. Formulas identified in at least four epilimnion samples that a) decrease (rho < 0) or b) increase (rho > 0) with time as determined by Spearman rank analysis. Boxes correspond to 1) protein-, 2) lignin- ,and 3) tannin-like formulas [Minor et al., 2014].

Figure 4. Identified formulas classified as a) photodegraded, b) photoproducts, c) microbially-degraded, and d) nonreactive based on correlations to chlorophyll and light intensity [Herzsprung et al., 2020]. Only formulas identified in all five surface samples are considered. Boxes correspond to 1) protein-, 2) lignin-, and 3) tannin-like formulas [Minor et al., 2014].

Figure 5. a) Temperature, b) [DO], c) [DOC], d) $SUVA_{254}$, e) $E_2:E_3$, and f) A_{254} measured during summer stratification (August 11th) and after fall turnover (November 3rd) at depth-discrete intervals. Analogous data for other sampling dates is provided in **Figures S21 – S26**.

Figure 6. Variations in a) % CHO, b) % N-containing, c) % S-containing, d) $H:C_w$, e) $O:C_w$, and f) DBE_w of formulas identified by FT-ICR MS with depth. Only dates which have a sample collected from above and below the thermocline are included in this plot.

Figure 7. Identified formulas that a) decrease (rho < 0) or b) increase (rho > 0) with depth in the depth-discrete samples analyzed with FT-ICR MS. Only those formulas detected in at least 15 out of 20 samples are considered in this analysis. Boxes correspond to 1) protein-, 2) lignin- and 3) tannin-like formulas [Minor et al., 2014].

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